Peptides as Radiopharmaceuticals: CMC Perspectives

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Outline

- Definitions
- Radiopharmaceutical Drugs
  - Monoclonal Antibodies (Ab), including various modifications
  - Peptides and Proteins
- CMC related Considerations
  - Ab based radiopharmaceuticals
  - Peptide based radiopharmaceuticals
    - Starting materials for peptide, characterization, specifications and safety.

Focus of the talk:

Chemically synthesized peptide radiopharmaceuticals (with $\leq$ 40 amino acids, for injectable solutions)

Definitions

- **Peptide** – any alpha amino acid polymer with specific defined sequence that is 40 amino acids or less in size
- **Chemically synthesized polypeptide** – any alpha amino acid polymer that is (a) made entirely by chemical synthesis, and (b) is less than 100 amino acids
- **Protein** – any alpha amino acid polymer with a defined sequence that is greater than 40 amino acids in size
- **Biological product** – a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein (except any chemically synthesized polypeptide, or analogous product .....

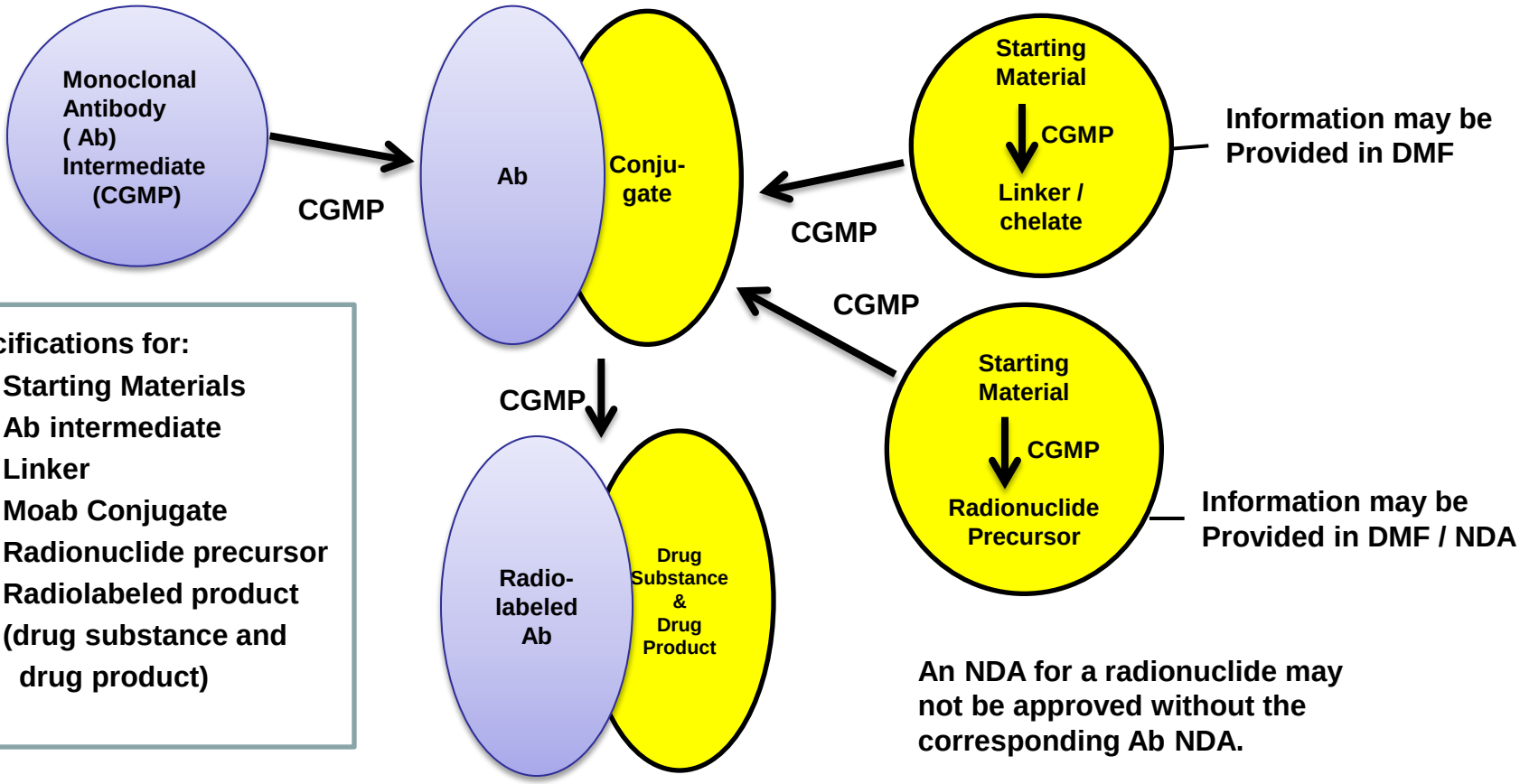
Radionuclide Examples

Radio-nuclide	^{89}Zr	^{177}Lu	^{68}Ga	^{90}Y	^{111}In	^{64}Cu	^{131}I	^{18}F
Chemical Form	^{89}Zr-oxalate	$^{177}LuCl_3$	$^{68}GaCl_3$	$^{90}YCl_3$	$^{111}InCl_3$	$^{64}CuCl_2$	$Na^{131}I$	$Na^{18}F$
Production	Cyclotron	Reactor	Generator	Reactor	Cyclotron	Cyclotron	Reactor	Cyclotron
Half-Life	3.3 days	6.73 days	68 Minutes	2.67 days	2.8 days	12.7 Hrs.	8.02 days	109.77 minutes
Decay Product	^{89}Y	^{177}Hf	^{68}Zn	^{90}Zr	^{111}Cd	^{64}Zn; ^{64}Ni	^{131}Xe	^{18}F

Ab Based Radiopharmaceuticals

- Radiolabeling of Ab with conjugated bifunctional chelate (e.g., with ^{90}Y, ^{89}Zr)
- Direct radiolabeling of Ab (e.g., with ^{131}I)
- Other methods.

Antibody Based Radiopharmaceutical



Specifications for:

- **Starting Materials**
- **Ab intermediate**
- **Linker**
- **Moab Conjugate**
- **Radionuclide precursor**
- **Radiolabeled product (drug substance and drug product)**

An NDA for a radionuclide may not be approved without the corresponding Ab NDA.

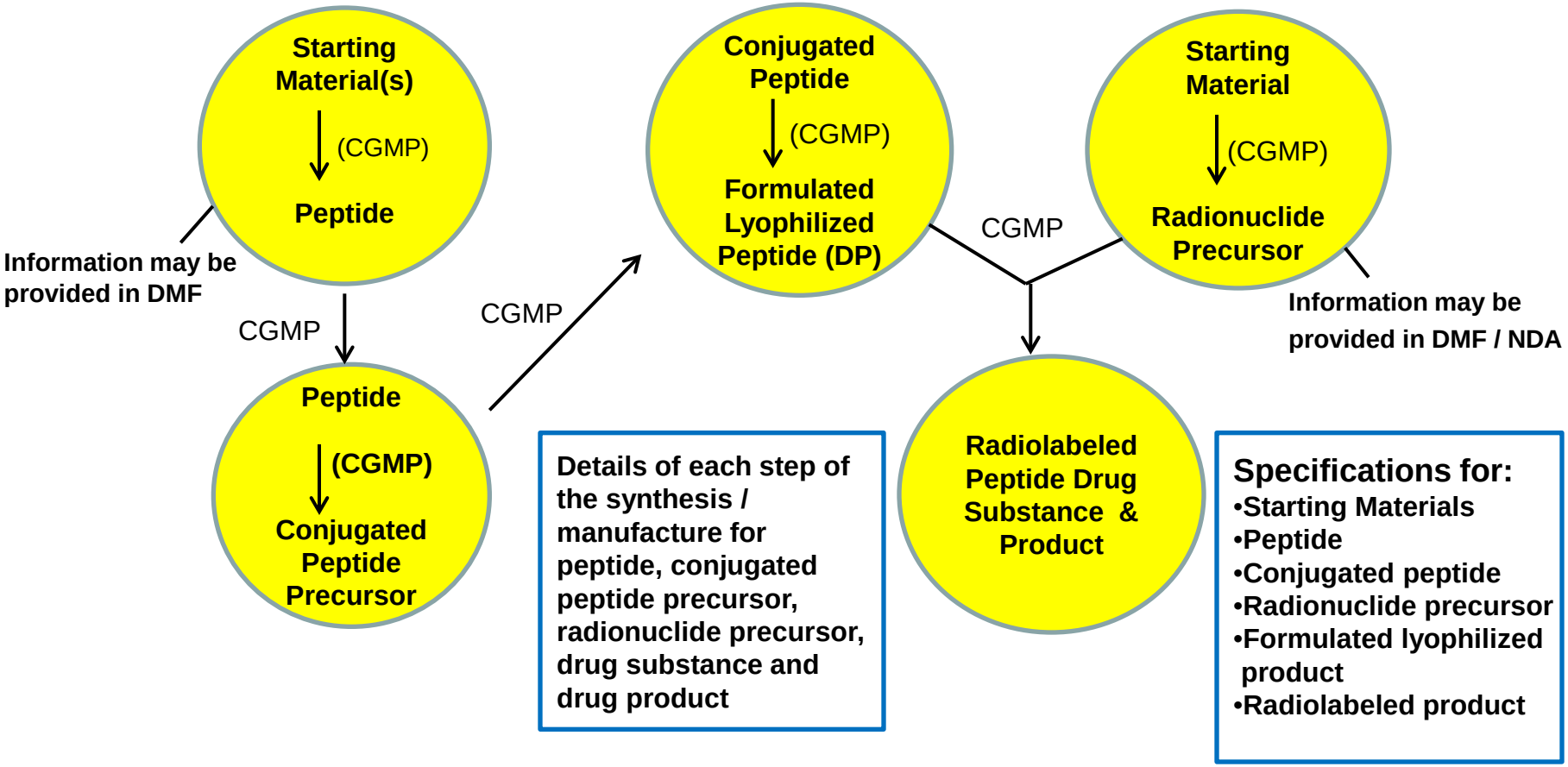
Ab Based Radiopharmaceuticals-Considerations

- Preservation of antibody characteristics:
  - Ab ⟶ Ab-Conjugate ⟶ Radiolabeled Ab.
  - Need adequate characterization (primary and higher order structures).
- Reproducibility of conjugation and radiolabeling reaction
  - Ab – linker / chelate ratio
  - Characterization of site of attachment of linker / chelate
  - Ab – radioisotope ratio; amount of naked antibody
- Impurity control at each stage of the process
  - e.g., free linker / chelate, other protein related impurities, aggregates, etc.
- IND - Relationship of preclinical batches to the clinical batch(es)
- NDA - Relationship of clinical batches to the commercial product

Peptide Radiopharmaceuticals

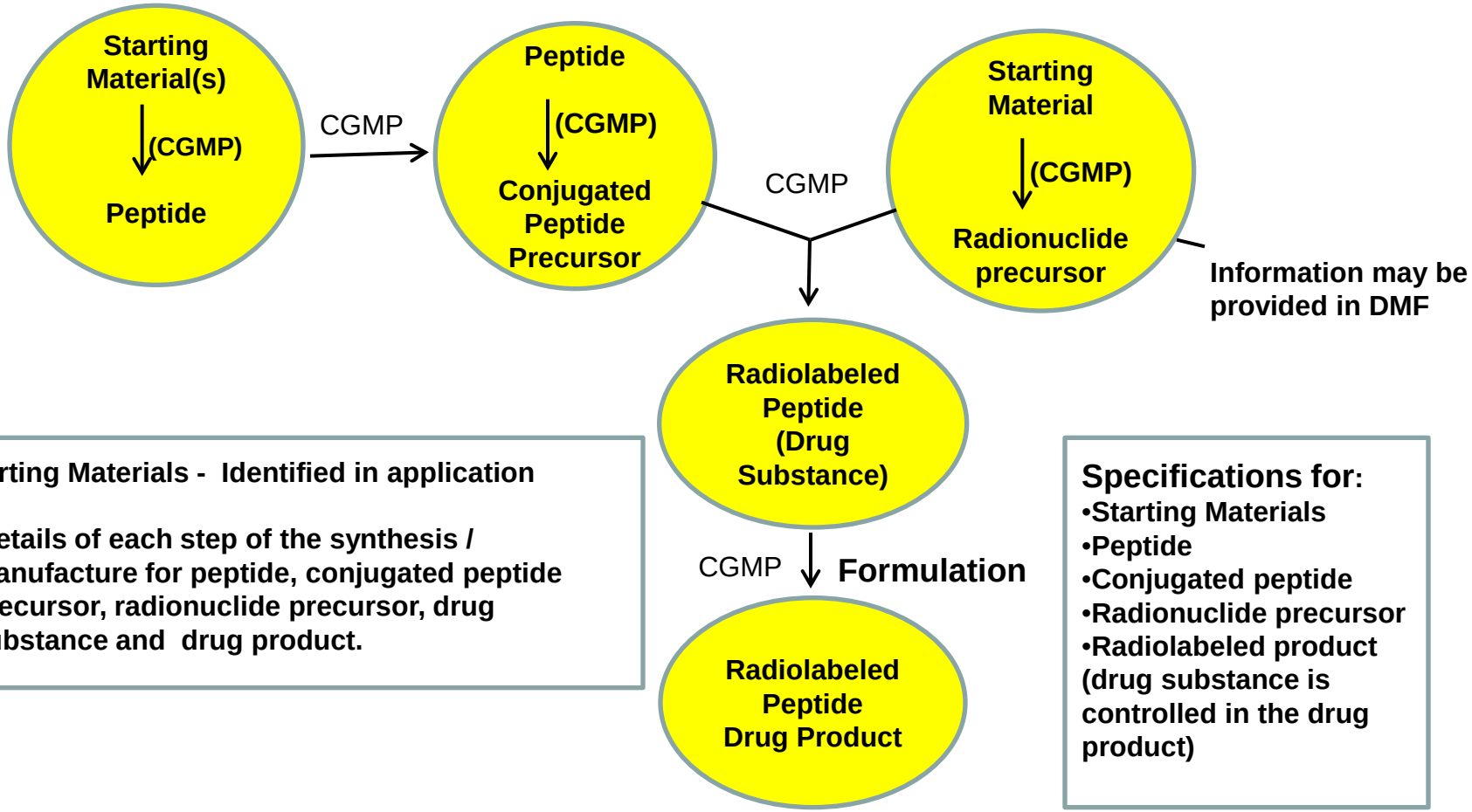
- Radiolabeling of peptide with conjugated bifunctional chelate (e.g., ^{89}Zr, ^{68}Ga, ^{111} In)
  - Lyophilized powder formulation (for injection)
  - Solution formulation (injection)
- Direct radiolabeling of peptide (e.g.,^{18}F, ^{131}I)

Lyophilized Powder Formulation



An NDA for a radionuclide may not be approved without the "Kit for the peptide" NDA.

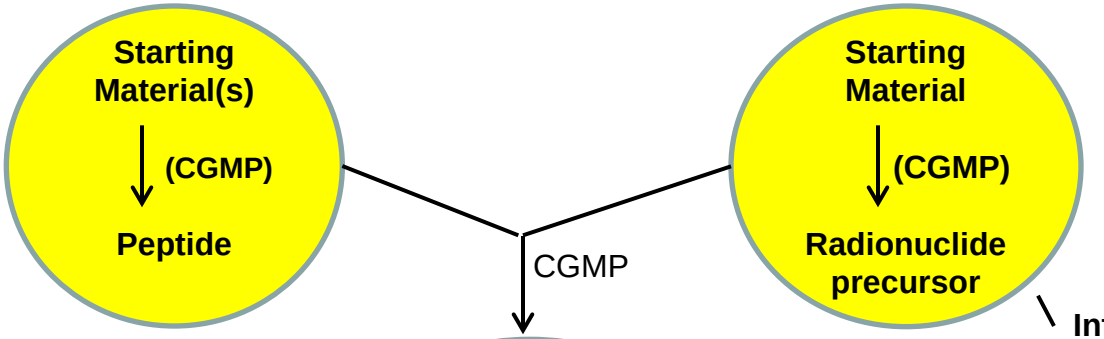
Solution Formulation (Injection)



- **Starting Materials - Identified in application**
- **Details of each step of the synthesis / manufacture for peptide, conjugated peptide precursor, radionuclide precursor, drug substance and drug product.**

Specifications for:
- **Starting Materials**
- **Peptide**
- **Conjugated peptide**
- **Radionuclide precursor**
- **Radiolabeled product (drug substance is controlled in the drug product)**

Direct Radiolabeling of Peptide



Starting Materials - Identified in application

Details of each step of the synthesis / manufacture for peptide, radionuclide precursor, drug substance and drug product

Specifications for:
- **Starting Materials**
- **Peptide**
- **Radionuclide precursor**
- **Radiolabeled product (drug substance is controlled in the drug product)**

Considerations For Starting Materials for Peptide

- Amino acids and derivatives
  - Name and address of the vendor
  - Specifications – identification, assay, optical rotation, chemical and **chiral purity**, impurities, water content, microbial control (if appropriate).
  - Certificate of Analyses (COA) with test results.
  - Uncommon amino acid derivatives / use of novel synthesis method
    - Provide details of synthesis and characterization

Peptide and Peptide Conjugate Characterization

- Primary structure – amino acid sequence (method of synthesis)
- Use a combination of analytical methods
  - Amino acid analysis, Edman degradation, MS, CD, fluorescence spectroscopy, fragment mapping, Ellman test (for disulfides), NMR, test to check chirality of the amino acid
- Higher order structure characterization and/or in vitro bioassay which is relevant to the mechanism of action may be needed.
- Information and characterization on potential peptide related impurities that can arise from peptide synthesis, cyclization (if cyclic peptide), and degradation (oxidation, deamidation, hydrolysis, isomerization)
  - Mainly for addressing immunogenicity concerns of certain peptide drugs
  - Important to choose a supplier carefully to minimize differences in impurities (e.g., D-amino acids)

Controls for Peptide and Peptide Conjugate

- Well characterized reference standard for peptide, peptide conjugate and drug substance (non-radioactive)
- Generally applicable tests;
  - Appearance
  - Peptide identity by amino acid analysis, HPLC/MS (identity of conjugate)
  - Purity, individual impurities (e.g., free conjugate molecule or related impurities), largest single unknown impurity, and total impurities by HPLC (HPLC method discriminative in detecting all potential related impurities- D amino acids)
  - Peptide assay by HPLC (or by quantitative aa analyses), Water content, residual solvents, inorganic impurities (ICP analysis)
  - Counter ion content, other relevant ionic impurities
  - Specific optical rotation
  - Bioassay
  - Microbiological attributes

Radiolabeled Peptide Attributes

- Appearance
- Identity - Identity of radiolabeled peptide; Identity of radionuclide
- Identity of higher order structure and/or in vitro bioassay which is relevant to the mechanism of action
- Purity - Radiochemical purity; Chemical purity
- Specific activity
  - Mass of radiolabeled peptide; mass of unlabeled peptide
- Assay of radioactivity
- pH
- Osmolality
- Impurities (specified, unspecified and total)
  - Peptide related impurities, degradation products, residual solvents (as appropriate)
- Identity and assay of functional excipients (e.g., antimicrobials, stabilizers)
- Microbiological attributes (sterility, bacterial endotoxins, etc.)

Safety Considerations

- Immunogenicity
  - Peptide, proteins, antibodies
  - Related impurities
- Peptide excipient interactions – safety and potency
- Guidance for Industry -Immunogenicity Assessment for Therapeutic Protein Products

  http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM338856.pdf

Conclusion

- Various radiopharmaceutical drugs
  - Peptides, Proteins, Antibodies
- Considerations for peptide radiopharmaceuticals
  - Starting materials
  - Characterization,
  - Attributes to test
  - Safety.

Thank-you

SUPPLEMENTARY MATERIAL

Drug Master Files

- When referencing a DMF, you must include a letter of authorization (LOA) obtained from DMF holder in your application.
- Identify what the DMF is being referenced for.
  - Type II - Drug substance, drug substance intermediate, and materials used in their preparation, or drug product
    - Examples - Radionuclide (including generator produced radionuclide), Precursor, Synthesizer cassettes, etc.
  - Type III - Packaging materials
  - Type IV - Excipient, colorant, flavor, essence, or materials used in their preparation
  - Type V - FDA accepted reference information (You must get permission to submit type V DMF) http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/DrugMasterFilesDMFs/default.htm